EVALUATION OF SERUM MAGNESIUM LEVEL IN TYPE 2 DIABETES MELLITUS AND IT'S COMPLICATIONS

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CERTIFICATE

This is to certify that this dissertation in **"EVALUATION OF SERUM MAGNESIUM LEVEL IN TYPE 2 DIABETES MELLITUS AND IT'S COMPLICATIONS"** is a work done by **Dr.POONAM AGRAWAL,** under my guidance during the period 2004 - 2007. This has been submitted in partial fulfillment of the award of M.D. Degree in Biochemistry, (Branch - XIII) by the Tamil Nadu Dr.M.G.R. Medical University, Chennai - 600 032.

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CONTENTS

INTRODUCTION

Diabetes mellitus is a metabolic disease of growing concern not only because of it's adverse effect on various metabolism of the body, but also because it puts the patient at higher risk of developing various macro and microvascular complications like cardiovascular disease (Ischaemic heart - disease), cerebrovascular disease, peripheral arterial disease, retinopathy, nephropathy, neuropathy etc.

Low serum magnesium has been proposed as a risk factor not only for the development of Diabetes mellitus but also has been linked to the emergence of it's various micro and macrovascular complications.

In some studies, diabetes mellitus has been found to lead to loss of magnesium in the urine, associated with glycosuria, which further lowers the magnesium in the plasma of Diabetic patients, aggravating the risk for development of it's complications. But various studies on human and animal model has given contradictory results regarding the association of low magnesium and various macro and microvascular complication of DM.

Since the prevalence of DM is found to increase very fast, the interest developed to determine the actual level of magnesium in Type 2 Diabetic Mellitus and it's complications, and to asscertain how far it correlates with the established biochemical parameter of this metabolic diseases and whether it's determination could be a helpful indicator in assessing the development and intensity of it's complications.

Hence with the above view this work **"Evaluation of Serum Magnesium Level in Type 2 Diabetes Mellitus and it's Complications"** has been taken up for the study.

MAGNESIUM

Magnesium is one of the most plentiful element on the earth. It occupies a position in group IIA in periodic table with atomic number 12 and atomic mass 24.31.

In vertebrates it is the fourth most abundant cation and the second most abundant intracellular cation[1].

A healthy adult human body (70 kg) contains 25 gm of magnesium.

SOURCES

Food items and beverges rich in magnesium are tabulated in Table No.1.

In addition, hard water is a substantial source of magnesium. Estimated daily intake of 2.3 mg and 5.21 mg of magnesium in subjects residing in soft and hard water areas respectively, have been reported, based on adults who consume 2 liter of water daily[30].

ABSORPTION

Magnesium is absorbed along the entire intestinal tract, including large and small bowel, but the site for maximal magnesium absorption appears to be distal jejunum and ileum[34,35,36] . Intestinal magnesium absorption is 30% to 50% under normal dietary condition[34,35,36]. Absorption of magnesium is inversely proportional to the amount ingested[37,38]; for example 65% of magnesium is absorbed with an intake of 36 mg versus only 11% absoprtion with an intake of 973 mg of magnesium[39].

There appears to be both an unsaturable passive and saturable active transport system for magnesium absorption, which may account for higher fractional absorption at low dietary magnesium intake.

Factors Influencing Magnesium Absorption

Factors enhancing and factors interfering magnesium absorption are given in Table No.2.

DISTRIBUTION OF MAGNESIUM

Out of total 25 gms of magnesium present in adult human body, 53% is found in bones, 27% is found in muscle, 19.2% is found in soft tissues and 0.8% is found in circulation. Out of 0.8% mg present in circulation, 0.5% is present inside the RBCs and 0.3% is present in the serum[2,3,4,5].

Distribution of total magnesium in the body is given in Table No.3.

Magnesium occurs in higher concentration in intracellular compartment as compared to extracellular. Total intracellular magnesium concentration has been reported to range between 5-20 mM[6,7], which is present either in free form or is combined with ATP. Only 0.5 to 5% of total intracellular magnesium is said to be present in free form depending upon the cell type and the means of measurement, the rest of it is combined with ATP[8].

It has been found that, it is the free form of magnesium inside the cell, that is important for enzymatic actvity[9,10].

Extracellular magnesium serves to maintain intracellular magnesium[11,12,13]. However, in the studies of Reinhart R.A. et al., Whang R. et al., Whang, R., Elin R.J., et al., Marx J.J. et al., Gunther, T, it has been found

that there is no good correlation between serum magnesium and intracellular magnesium level[14,15,17-20].

Various theories have been put forward to explain the magnesium transport inside and outside the cell. They are :

1. Magnesium transport into and out of the cell takes place due to presence of carrier mediated transport system, possibly regulated by concentration of Mg^{++} within the cell[20,21].

2. The efflux of magnesium ion from cell appear to be coupled to sodium transport and requires energy[22,23]. Efflux of Mg^{++} is coupled to the movement of Na^{+} into the cell down to it's electrochemical gradient. Maintenance of this process requires the subsequent extrusion of Na^{+} by Na^{+} K^{+} ATPase.

3. There is also evidence for a Na^{+} independent efflux of Mg^{++}.

4. Magnesium influx also appears to be linked to Na^{+} and HCO_3^{-} transport but by a different mechanism than efflux[20,24].

Factors affecting magnesium transport into and out of the cells consist of pharmacological agents and harmones which are summarised below :-

Pharmacological agent like β-agonist is found to stimulate magnesium influx, but has no effect on it's efflux. Grubbs R.D. has stated that Epidermal Growth Factor increases magnesium transport into the vascular smooth muscle cell line[25].

According to Lostroh A.J., et al., Krahl M.E., insulin and dextrose increases ^{28}Mg uptake by a number of tissues including skeletal and cardiac muscle[26].

Kumar D; et al., Barbagallo M, et al., and Hwang D.L., et al., have all stated that insulin increases Mg^{++} in human red blood cells, platelets and lymphocytes[27,28,29].

Both Type 1 and type 2 DM patients have low intracellular Mg level, because of lower level of insulin in former group and because of resistance of insulin action in the later group[1].

EXCRETION OF MAGNESIUM

The major excretory pathway for absorbed magnesium is through the kidney. The kidneys are the main organs of magnesium homeostasis in maintaining plasma concentration. Only 3 to 6% of filtered load of magnesium in the kidney is excreted which works out to about 3.6 - 20.7 mg/day (3-17 meq day)[11]. Approximately 25-30% of filtered magnesium is reabsorbed in proximal tubule and 60-75% in ascending limb of the loop of Henle[43]. Only about 2-5% is reabsorbed at the distal convoluted tubule. It is said that tubular secretion of magnesium does not occur[155].

Reabsorption of magnesium in distal tubule is load dependent. There is evidence for harmonal regulation of renal clearance of magnesium. Aldosterone and parathyroid harmone (PTH) are two harmones which influence magnesium excretion in urine. Aldosterone increases urinary excretion of magnesium. PTH has got negative feedback control in magnesium homeostasis. Hypomagnesemia enhances PTH secretion and PTH in turn enhances tubular reabsorption of magnesium[156]. Certain drugs are said to enhance the urinary loss of magnesium, a list of which is given in Table No.4[88]. About 25 to 50 mg of endogenous magnesium may be excreted daily in faeces[44].

Magnesium may also be lost in the sweat, in amounts estimated at approximately 15 mg / day[45]. Though text book description of 0.9 mg per day loss is also available[11].

FUNCTIONS OF MAGNESIUM IN HUMAN BODY

1. Role of Magnesium Present Intracellularly

Intracellular magnesium is important for over 300 different enzymes reactions either as a structural cofactor or an allosteric activator of enzyme activity[45,52,53].

In ATP, magnesium binds to phosphate group, thereby forming a complex that assists in transfer of ATP phosphate. A list of magnesium dependent enzymes, their substrates and products are given in Table No.5.

2. Role of Magnesium Present Extracellularly

a) Extracellular magnesium serves as a source for maintaining intracellular magnesium[9].

b) Extracellular magnesium has also been shown to stabilize the nerve axon. Lowering the serum magnesium concentration decreases the thresold of axonal stimulation and increases nerve conduction velocity.

(c) Extracellular magnesium also influences release of neurotransmitters at neuromuscular junction by competitively inhibiting the entry of Ca^{++} in presynaptic nerve terminal. Low serum magnesium increases neuromuscular excitability.

3. Role of Magnesium Present in Bone

Nearly two third of skeletal magnesium is incorporated into mineral lattice of the skeleton. About one third is elutable from bone and therefore exchangeable with ECF. It is this fraction of bone magnesium that is thought to serve as a reservoir for maintaining a normal concentration of magnesium in the plasma.

4. Role of Magnesium in Membrane Function

Magnesium is a cofactor for two active ion transport system across membranes requiring ATP, namely Na^+ K^+ ATPase and Ca^{++} ATPase[162,163].

5. Role of Magnesium on Insulin Secretion

Magnesium is a factor important for insulin secretion and insulin action. Magnesium depletion per se has been reported to impair insulin secretion and decrease peripheral insulin sensitivity[143] and could contribute to diminished insulin effects.

6. Role of Magnesium in Skeletal Muscle Function

Magnesium within muscle cell in said to interfere with the action of calcium, which is necessary for regulation of contraction and relaxation of the myofibril[160]. Effect on magnesium on muscle contraction, by interfering action of calcium can be explained as follows :

1. Magnesium inhibits release of calcium from Sarcoplasmic Reticulum in response to increased influx of calcium from extracellular site, the effect which leads to relaxation of the myofibril[160,161].

2. In addition, magnesium activates the Ca^{++} - ATPase pump of Sarcoplasmic Reticulum which decreases calcium concentration in the Sarcoplasm.

3. Further, magnesium competes with calcium for specific binding sites on troponin C and myosin. Thus magnesium interferes with initiation of muscle contraction which is brought about by the binding of calcium to muscle protein[157,159].

   All the above effect of magnesium within the muscle cell causes a relaxation of the myofibril and decreases skeletal muscle tone and tension.

7. Role of Magnesium in Smooth Muscle Contraction

Calcium binding in a smooth muscle cells initates Acetylcholine release and smooth muscle contraction. But magnesium binding to the calcium sites prevents calcium binding and inhibits contraction[158].

8. Role of Magnesium on Cardiovascular Functions

a. Magnesium is said to inhibit - platelet adhesions and aggregation by stimulating the release of Prostacyclins from endothelium of blood vessels[164,165].

b. Magnesium reduces the likelihood of arrythmia by dilating the coronary arteries which enhances the perfusion of myocardium[166,168].

c. Magnesium decreases total and LDL cholesterol but increases HDL cholesterol[169]. That way plays a role in determining the luminal size of coronary vessles.

d. Extracellular and membrane bound magnesium in vascular smooth muscle cells, control and regulate the entry of calcium into the cells and it's binding and distribution within the cell[170]. It also stimulates the release of prostaglandins from the vascular endothelium[171], as a result of which the tone, contractility and reactivity of vascular smooth muscle especially that of myocardial, renal, placental and cerebral vessels are influenced by magnesium[172-174].

9. Role of Magnesium on Blood Clotting

In blood coagulation, calcium and magnesium are antagonistic, with calcium promoting the clotting process and magnesium inhibiting it[175].

Reference range of serum magnesium in humans is given in Table No.6.

EFFECTS DUE TO VARIATION IN THE LEVEL OF SERUM MAGNESIUM

Hypomagnesemia

Serum magnesium value less than 1.83 mg/dl (1.5 mEq/L) usually indicate magnesium deficiency[37, 176-178].

Though magnesium content of peripheral lymphocyte is found to correlate with skeletal and cardiac muscle magnesium content, and it's measurement seems to be more accurate indicator of magnesium status than serum magnesium concentration;[179-181] it is serum magnesium concentration, which is most available and commonly employed test to assess magnesium status in the body.

In one study, Wong E.T. et al., have reported that approximately 10% of patients admitted to large city hospitals have Hypomagnesemia[177].

In another study, Ryzen E. et al., reported 65% of the patients to be hypomagnesemic among ICU admissions[181,182].

Clinically apparent hypomagnesemia or magnesium depletion is usually due to loss of magnesium from either GIT or Kidney[184].

Causes for magnesium defeciency is enlisted in Table No.7.[183]

Clinical sequelae of magnesium depletion : Frequent menifestations of moderate to severe magnesium defecieicny are shown in Table No.8.

Hypermagnesemia

Wong E.T et al., who have observed as many as 10% of hospitalized patients to be hypomagnesemic, have also observed, as many as 12% of hospitalized patients to have mild or moderate elevation in Sr. magnesium concentration[177].

Causes for hypermagnesium is enlisted in Table No.9.

The clinical menifestation at different level of hypermagnesemia is enlisted in Table No.10.

RECOMMENDED DAILY ALLOWANCES

RDA for various age groups in given in Table No.11.

DIABETES MELLITUS

Defenition

Diabetes Mellitus is a group of metabolic disease which is characterized by hyperglycemia, resulting from defect in insulin secretion, insulin action or both[55].

Prevalence

Currently number of diabetic patients worldwide is estimated to be around 150 million, two third of which are residing in developing countries[56].

This number is predicted to double by 2025, with the greatest number of cases in India and china alone[57].

Symptoms of Diabetes Mellitus

The classic symptoms of Diabetes Mellitus include Polyuria, Polydipsia, polyphagia and unexplained weight loss.

Diagnosis of Diabetes Mellitus

American Diabetic Association (ADA), Criteria for diagnosis of Diabetes Mellitus are given in Table No.12[55].

As per criteria of diagnosis of Diabetes Mellitus, since plasma glucose is elevated in this condition, it becomes essential to know it's homeostasis which is reviewed below :

Glucose Homeostasis

Normal glucose homeostasis is tightly regulated by three interrelated processes :-

1. Glucose production in the liver
2. Uptake and utilization of glucose by peripheral tissue
3. Insulin secretion

1. Glucose Production in the liver : The liver of well fed persons actively synthesize glycogen and triacylglycerol, such a liver is glycogenic, glycolytic and lipogenic. In contrast, that of the fasting person is glycogenolytic, glyconeogenic, ketogenic and proteolytic.

The liver is moved between these metabolic extremes by a variety of regulatory mechanisms : Substrate supply, allosteric effectors; covalent modification and induction - repression of enzymes[58].

2. Uptake and utilisation of glucose by peripheral tissues : Glucose transport into the cells is modulated by two families of proteins[43] :

i. Intestinal sodium / glucose cotransporter

ii. Facilitative glucose transporters (GLUT)

The intestinal sodium / glucose contransporter promote the uptake of glucose and galactose from the lumen of small bowel and their reabsorption from the urine in the kidney. The transporter uses the electrochemical Na gradient to transport glucose against it's concentration gradient.

The second family of glucose carriers, termed facilitative glucose transporters (GLUT) is located on the surface of all cells. These transporters are designated GLUT-1 to GLUT-7.

Distribution and function of these glucose transporters are enlisted in Table No.13.

3. Insulin Secretion : Insulin is a protein harmone secreted by β cells of islet of Langerhans' of the pancreas. It is the key harmone for regulation of blood glucose and generally normglycemia is maintained by balanced interplay between insulin secretion and insulin action.

CHEMISTRY OF INSULIN

Human insulin has M.W. of 5,743 Da and consists of 51 aminoacids in two chains (A and B). These two chains are joined by two disulfide bridges, with a third disulfide bridge within the A chain.

In most species location of disulfide bridges are invariable and interchain disulfide bridges connect `A'$_7$ to `B'$_7$ and `A$^{'}_{20}$ to `B'$_{19}$. Intrachain disulfide bridge connects residue 6 and 11 of the A chain. A chain have 21 amino acids and B chain have 30 amino acids respectively[43,46].

SYNTHESIS OF INSULIN

Insulin is synthesized by ribosomes of rough endoplasmic reticulum of pancreatic β cells in it's precursor form "Preproinsulin", a protein which has M.W. of 11,500 Da.

Preproinsulin has 23 amino acids long pre, or leader sequence which directs the molecule into the cisternae of endoplasmic reticulum and then this sequence is removed to result in proinsulin molecule having M.W of 9,000 Da.

Proinsulin molecule provides the conformation necessary for proper disulfide bridges. It varies in length from 78 to 86 amino acids, with the variation occurring in the length of C-peptide region.

Proinsulin is stored in secretary granules in the golgi complex of the β cells, where protolytic cleavage to insulin and connecting peptide (C-peptide) occurs[43].

RELEASE OF INSULIN

Various factors stimulating and inhibiting the release of insulin is presented in Table No.14.

Glucose is the most important stimulus for insulin release. Glucose elicits the release of insulin from pancreas in two phases. First phase begins 1 or 2 minutes after IV injection of glucose and ends within 10 minutes. This phase represents rapid release of "stored" insulin. Second phase begins at the point where 1st phase ends. This phase depends upon continuing insulin synthesis and release and lasts until normoglycemia has been restored; usually within 60 to 120 minutes[43].

Mechanism of glucose induced release of `presynthesized (stored)' insulin

Glucose is taken up by pancreatic β cells via GLUT-2. Glucose is phosphorylated by glucokinase and further degradation leads to the formation of pyruvate. This pyruvate forms ATP in the mitochondria of β cells of pancreas. ATP is necessary for the delivery of energy needed for the release of insulin, but it is also involved in the cell membrane depolarization. The ADP / ATP ratio leads to activation of sulphonylurea receptor - 1 (SUR-1) protein, which leads to closure of the adjacent potassium channel [Potassium inward rectifier (KIR) 6.2 Channel]. Closure of KIR 6.2 channel will alter the membrane potential and open calcium channels, which triggers the release of `preformed' insulin form its storage granules[51].

Diagramatic Representation of abovesaid process has been depicted in Figure No.1.

Other agents including intestinal harmones (gastrin, secretin, GIT polypeptide) and certain amino acids (Leucine and Arginine) as well as sulfonylureas stimulate insulin release alone, but have no effect on insulin synthesis[43].

Degradation

Liver is the major organ for insulin degradation. On the first pass through the portal circulation approximately 50% of the insulin is extracted by the liver where it is degraded. Kidney and placenta helps in additional degradation. Plasma half life of insulin is 3 - 5 minutes under normal conditions[46].

`Proteases' and `hepatic glutathione - insulin trans hydrogenases' are two enzyme system involved in insulin degradation.

PHYSIOLOGICAL ACTION OF INSULIN

A. EFFECT OF INSULIN ON GLUCOSE METABOLISM

a. Insulin effecting uptake of glucose

Insulin causes translocations of glucose transport protein (GLUTs) from the golgi apparatus to the plasma membrane, thus facilitating cellular uptake of glucose.

GLUT-4 present in striated muscle and adipose tissue is the major transprotein regulated by insulin[46].

GLUT-2 which is present on hepatocyte, β cell of pancreas and basolateral membranes of intestinal and renal epithelial cells, are insulin independent.

b. Insulin effecting utilisation of glucose

Insulin favours the utilization as well as storage of glucose. Insulin favours glycolysis by increasing the activity and amount of several key glycolytic enzymes (glucokinase, phosphofructokinase and pyruvate kinase).

Dephosphorylation of glycogen synthase activates this enzyme and leads to increase glycogen synthesis. Insulin favours dephosphorylated state of glycogen synthase by activating phosphodiesterase which decreases cAMP level. So in presence of insulin glycogen synthesis is enhanced.

Insulin also inhibits glycogenolysis by favouring the inactivation of glycogen phosphorylase and inhibiting the glucose - 6 - phosphatase.

Gluconeogensis is inhibited by insulin by repressing the key enzymes, especially pyruvate carboxylase, phosphenol pyruvate carboxykinase and glucose - 6 - phosphatase.

Net effect is lowering of blood glucose level.

B. EFFECT OF INSULIN ON LIPID METABOLISM

Insulin favours lipogensis, and inhibits lipolysis in adipose tissue.

C. EFFECT OF INSULIN ON PROTEIN METABOLISM

Protein synthesis is favoured and catabolism is inhibited.

MECHANISM OF ACTION OF INSULIN

Insulin action begins when this harmone binds to a specific glycoprotein receptor "Insulin receptor" on the surface of target cell.

The insulin receptor is a heterodimer consisting of two subunits, designated α and β, in configuration α_2 β_2, linked by disulfide bond. α subunit is entirely extracellular, and it binds to insulin, probably by cysteine rich domain. β - subunit is transmembrane protein that performs the second major function of a receptor i.e. signal transduction.

Cytoplasmic portion of β subunit has tyrosine kinase activity and autophosphorylation site. Both of these are thought to be involved in signal transduction and insulin action.

Half life of insulin receptor is 7 - 12 hours. Insulin receptors are found on most mammalian cells, in concentration of upto 20,000 per cell. When insulin binds to the receptor, several events occurs :

1. There is conformational change of the receptor
2. Receptors cross - link and form microaggregates
3. The receptor is internalized

&

4. One or more signals are generated

Various metabolic effects of insulin may be mediated by protein phosphorylation, dephosphorylation, effects on mRNA translation or affecting the gene expression.

Various Biochemical parameters useful in diagnosis and management of diabetes mellitus is given in Table No.15.

Classification of DM :

Main types of DM are as follows[55]:

a. Type 1 (I) or Juvenile onset Diabetes : It occurs because of β cell destruction, usually leading to absolute insulin defeciency :

It may be of two types :

i. Autoimmune type

ii. Idiopathic type

b. Type 2 (II) or adult onset Diabetes : This ranges from predominant insulin resistance, with relative insulin defeciency to a predominant insulin secretary defect, with or without insulin resistance.

Main feature for differentiation between there two groups is pathogenesis. Age of onset is not the criteria.

Other specific types of diabetes mellitus in given in Table No.16.[55]

PATHOGENESIS OF TYPE 2 DIABETES MELLITUS

The two metabolic defects that characterize type 2 diabetes mellitus are[47] :

1. A derangement of insulin secretion due to β cell dysfunction and
2. A decrease response of peripheral tissue to respond to the insulin (Insulin Resistance).

The metabolic defect leading to Type 2 DM are illustrated in Figure No.2.

A. BETA CELL DYSFUNCTION

Early in the course of Type 2 diabetes, insulin secretion appears to be normal and plasma insulin level is not reduced. However, normal pulsatile, oscillating pattern of insulin secretion is lost and the rapid first phase of insulin secretion triggered by glucose is obtunded. It shows derangement in β cell responses to hyperglycemia early in Type 2 diabetes mellitus.

Later in the course of Type 2 Diabetes Mellitus, a mild to moderate deficiency of insulin develops. Here irreversible β cell damage appears to be present, because of Glucose toxicity and, or Lipotoxicity.

a. Glucose Toxicity : The notion that hyperglycemia itself can decrease insulin secretion has led to concept of glucose toxicity, which implies the development of irreversible damage to cellular components of insulin production[48,49].

Reactive oxygen species (ROS) produced during oxidative glucose metabolism in β cells are normally detoxified by catalase and superoxide dismutase. β cells are equipped with a low amount of these proteins and also of

redox regulating enzyme glutathione peroxidase[48]. Hyperglycemia leads to large amount of ROS in β cells, with subsequent damage to cellular components.

b. Lipotoxicity : More recently the concept of lipotoxicity involving the β cells has been put forward[51]. There are several mechanism of lipotoxicity :

1. According to Robertson R.P. *et al.,* in the presence of glucose, fatty acid oxidation in β cells is inhibited and accumulation of long chain acyl coenzyme A occurs[50]. Long chain acyl coenzyme A can diminish the insulin secretary pathway by opening β cell K channel.

2. A second mechanism might be increased expression of uncoupling protein - 2 in presence of acyl Co A, which could lead to reduced ATP formation and hence decreased insulin secretion.

3. A third mechanism might involve apoptosis of β cell, probably via fatty acid or triacylglyserol induced ceramide synthesis, or generation of Nitric Oxide[51].

Role of **Islet Amyloid deposition** in causing β cell dysfunction is controversial.

B. INSULIN RESISTANCE

Insulin Resistance is said to be present when the biological effects of insulin are less than expected for both

* Glucose disposal in skeletal muscle and
* suppression of endogenous glucose production primarily in the liver[59].

Insulin Resistance may be due to a decrease in the number of insulin receptor or more importantly impairment in the postreceptor signalling of insulin receptors with or without decrease in the number of insulin receptors.

MECHANISM OF INSULIN RESISTANCE

i. Role of Phosphorylation and Dephosphorylation of Insulin receptor substrate (IRS) protein in Insulin Resistance

Normally, after insulin binds to it's receptor, it leads to tyrosine phosphorylation of insulin receptor substrate (IRS) proteins which serve as binding scaffolds for various adaptor proteins and leads to the downstream signalling cascade[60].

In state of insulin resistance, the positive effects on downstream responses exerted by tyrosine phorsphorylation of the receptor and IRS proteins are opposed by dephosphorylation of these tyrosine side chains by cellular protein - tyrosine phosphatase and by protein phosphorylation on serine and threonine residue[61].

Phosphotyrosine phosphatase 1B is widely expressed and has important role in the negative regulation of insulin signalling[62].

Serine and threonine phosphorylation of IRS - 1 reduces it's ability to act as a substrate for tyrosine kinase activity of the insulin receptor and inhibits it's coupling to it's major downstream effector systems.

Several IRS serine kinases have been identified, including various mitogen - activated protein kinases, C-Jun NH2 terminal kinase, atypical protein kinase C, and phosphatidylinositol 3' kinase[60].

ii. Role of Adipocyte products and inflammation

Increased concentration of NEFA and Inflammatory Cytokines (TNF-α, and IL-6) released by expanded adipose tissue adversly affects insulin signalling cascade[63,64].

NEFA inhibits insulin stimulated glucose metabolism in skeletal muscle and stimulate gluconeogenesis in liver[65,66].

TNF-α enhances adipocyte lipolysis, which further increases NEFA, and also elicits it's own direct negative effects on insulin signalling pathway[67]. IL-6 inhibits the insulin signal by augmenting the expression of suppressor of cytokine signalling (SOCS) protein. SOCS family of proteins participate in IRS protein degradation through a ubiquitin - proteosomal pathway.

iii. Adiponectin

Whereas NEFA and several adipokines are increased in viseral obesity, the concentration of the adipose specific protein adiponectin are decreased, reducing it's insulin sensitizing effect in liver and muscles[63,68].

COMPLICATIONS OF TYPE 2 DIABETES MELLITUS

Complications of Type 2 Diabetes Mellitus have been classified in Table No.17.

PATHOGENESIS OF DIABETIC COMPLICATIONS

Although clinical manifestations of diabetic complications (Microvascular / macrovascular) are very diverse, these syndromes share certain common pathophysiological charactersitics[93].

Chronic tissue damage in diabetes is generally related to the severity and duration of Hyperglycemia, other determinants of specific complications

include genetic predisposition, obesity, hypertension and dyslipidemia. Tissue damage may continue even after hyperglycemia has been improved (Hyperglycemia Memory[93])

ROLE OF HYPERGLYCEMIA IN DIABETIC COMPLICATIONS

The cause of most diabetic complications is probably prolonged exposure to high glucose level[93].

DCCT (Diabetic Control and Complication Trial), UKPDS (United Kingdom Prospective Diabetic Study) and Kumamato Study all have supported the idea that chronic hyperglycemia plays a causative role in the pathogensis of diabetic microvascular complication. According to these studies there was a non significant trend in the incidence of macrovascular complication[94].

MECHANISM OF HYPERGLYCEMIA INDUCED DAMAGE

Four major hypothesis which are not mutually exclusive, have been proposed to explain how hyperglycemia might lead to the chronic complication of diabetes mellitus. They are[93,94] :

A. Increased intracellular AGE formation

B. Increased polyol pathway

C. Increased protein kinase C activation

D. Increased hexosamine pathway.

A. Increased intracellular AGE formation

One theory is that increased intracellular glucose leads to the formation of advanced glycosylation end products (AGEs) via the nonenzymatic glycosylation of intracellular proteins. Nonezymatic glycosylation is the process which occur physiologically. In this process glucose and other

glycating compound e.g. dicarbonyl such as 3-deoxy glucosone, methylglyoxal and glyoxal chemically attach to amino group and other long lasting molecule such as nucleic acid without aid of enzyme[93,47].

These glycated protein undergo progressive dehydration, cyclization, oxidation and rearrangements to form AGE product[95].

AGEs have been shown to cross link proteins (e.g. collagen, extracellular matrix proteins), accelerate atherosclerosis, promote glomerular dysfunction, reduce nitric oxide (NO) synthesis, induce endothelial dysfunction and alter extracellular matrix composition and structure[94].

The formation of reversible and irreversible AGE is depicted in Fig.3.

B. Increased Polyol Pathway Flux

A second theory is based on the observation that hyperglycemia increases glucose metabolism via sorbitol pathway. When intracellular glucose is increased, some glucose is converted to sorbitol by the enzyme aldose reductase. Several mechanism has been proposed to explain how hyperglycemia induced increase in polyol pathway flux could damage the tissue involved :

These include[93] :

1. Sorbitol induced osmotic stress.

2. Decreased cytosolic Na^+ / K^+ ATPase activity.

3. Reduced cytosolic NADPH (thus increased oxidative stress within the cell).

4. Increase in cytosolic ratio of NADH / NAD+, thereby inhibiting activity of the enzyme Glyseraldehyde- 3-Phosphate dehydrogenase (GAPDH) and thus increasing intracellular concentration of triose phosphate[96]. Increased triose phosphate level could increase formation of both methylglyoxal, a highly active glycating compound and a precursor of AGE formation and (Via α-glycerol-3-phosphate) enhance production of DAG which activate protein kinase C. This polyol pathway which is normally inactive, and gets activated only when intracellular glucose level increases, has been depicted in Fig.No.4.

C. Increased `Protein kinase C Activation'

Koya D.et al., Craven PA et al., and Shiba T et al., have all reported that enhanced de-novo synthesis of dicylglyserol (DAG), leads to persistent and excessive activation of protein kinase C (PKC). De-novo synthesis of DAG is enhanced within the cell, because of enhance glucose flux through the glycolytic pathway in conditions of increase intracellular glucose[97,98,99].

In addition, Nishikawa T, et al., has proposed that increased cytosolic NADH / NAD+ associated with sorbitol oxidation to fructose and the inhibition of GAPDH by intracellular ROS generated in mitochondria could divert glysceraldehyde-3-PO_4 away from the glycolytic route and towards production of dihydroxy acetone phosphate (DHAP) and DAG[100].

Further, Scivittaro V et al., have suggested that the enhanced activity of PKC enzyme could also result from the interaction between AGEs and their cell surface receptor[101].

Among other actions, PKC alters the transcription of gene for fibronectin, Type IV collagen, contractile protein and extra cellular matrix proteins in endothelial cells and neurons.

D. Increase hexosamine pathway

Sayeski PP et al., Kolm Litty V et al., Daniels MC et al., all have proposed that hyperglycemia could cause diabetic complication by shunting glucose into the hexosamine pathway[102,103,104].

Hence Fructose 6 - PO4 is diverted from glycolysis to form Glucosamine - 6 - PO_4 which gets converted to UDP-N-acetyl glucosamine (UDP-Glc NAC) in the cytosol which can glycate transcription factors and thus enhance transcription of gene including plasminogen activator inhibitor -1 (PAI-1) and transforming growth factor β_1 (TGF - β_1). The glucosamine pathway is illustrated in Fig. No.5.

The above four possible mechanism involved in the development of chronic complication of DM have been illustrated in Fig. No.6.

Other than the mechanisms elaborated above, the hypothesis of mitochondrial superoxide production and it's association with diabetes mellitus has also been postulated.

Mitochondrial Superoxide Production : A unifying hypothesis

Nishikawa T. et al., has proposed the hypothesis that all four different pathogenic mechanisms described above can stem from a single hyperglycemia induced process, namely overproduction of superoxide by mitochondrial Electron Transport Chain (ETC)[100].

Wallace D.C. has supported this hypothesis via his experiment on cultured bovine aortic endothelial cells where he has observed that high glucose level increases ROS production inside the endothelial cells[106].

Other than **hyperglysemia** the risk factors associated with development of diabetic complications are **obesity, hypertension** and **dyslipidemia**.

Obesity

According to Mokdad AH et al., and Knowler WC et al., obesity is a common problem among diabetics. They have estimated that approximately 60% of Type 2 diabetes mellitus patients are obese[69,70].

The central distribution of fat and history of weight gain, in addition to body mass are independent risks of developing diabetic mellitus. Obesity in patients with Type 2 DM contributes to the development of complications like cardiovascular complications although the precise cause of increased cardiovascular morbidity and mortality in obesity is not known. According to Despres J.P. et al., obesity leads to development of insulin resistance and hyperinsulinemia[71].

Hypertension

Barrett - Connor E. et al., Modon M. et al., Lesse GP et al., Skyler J.S. et al.,[72,73,74,75], have all stated that diabetic patients have high blood pressure, independent of age or the presence of obesity or renal disease.

NHANES II data showed that hypertension is more than twice as prevalent among patients with type 2 DM than among those with normal glucose tolerance[76]. As per Krolewski A.S. et al., the prevalence of hypertension increases with the duration of diabetic mellitus[77].

Several studies have demonstrated that hypertension is a significant risk factor for the development of vascular disease in individual with diabetic mellitus[78]. According to Kuller LH et al., Tuck M.L. et al., hypertension is a prime risk factor for development of cardiovascular disease, cerebrovascular disease and peripheral vascular disease, when accompanies diabetes.

Dyslipidemia

Abnormalities of plasma lipid and lipoprotein metabolism - like hypertriglyseridemia, decrease HDL concentration, increase total cholesterol and decrease LDL cholesterol concentration are very common in diabetes and have long been thought to increase cardiovascular risk as they do in nondiabetic state.

DM not only changes lipoprotein concentration but also induces a number of alteration in lipoprotein composition that may influence atherosclerosis process; and may lead to macrovascular complications.

Small, dense LDL particles which is a form of LDL, is found to be associated with not only insulin resistance syndrome[81], but also has been demonstrated to be more susceptible to glycation and oxidation.

Apo B of LDL is glycated[82] and these glycated LDL is taken up by macrophages to form Foam cells. Not only glycation, but oxidation of LDL also is believed to play an important role in atherogenesis. Hunt J.V. et al., have shown that glycated LDL is more susceptible to oxidation[83].

Oxidation of LDL leads not only to increased foam cell formation[84,85,] but also increases adhesion of monocyte to endothelial cells.

It is also said that oxidised LDL also stimulates monocyte chemotaxis, and can be directly toxic to endothelial cells[86].

In addition, Lopes Virella MF et al., have shown that modified Lipoprotein species can be immunogenic and circulating immune complex may accelerate atherosclerosis[87].

Diabetic individuals have smaller proportion of HDL-2 subfraction and greater proportions of HDL-3, a distribution which is associated with atherosclerosis[78].

Other factors associated with Atherosclerosis in DM

1. Glycation of other protein like collagen.

2. Endothelial dysfunction : Nitric oxide (NO) synthase is endothelium derived and is the key regulator of vascular tone. According to Sobrevia L et al. NO synthase is impaired in DM[89].

Mortality is Diabetic Patients[92]

Death is usually due to complications in DM. The causes of death in diabetic patients in India and developed countries have been depicted in Figure No.7.

Having elaborated about the various complications of DM and the mechanism involved in pathogenesis of these complications, review on the complications micro and macrovascular, taken up for the study namely diabetic retinopathy, diabetic nephropathy, diabetic coronary atherosclerosis and diabetic peripheral vascular disease is as follows :

DIABETIC RETINOPATHY

Diabetic Retinopathy is a significant cause of blindness in diabetic patients.

Klein R. et al., in their population based study `Wincosin Epidemiologic study of diabetic retinopathy (WESDR) have stated that among type 2 DM patients suffering from the condition for less than 5 years duration, have retinopathy in 40% of the patient taking insulin and in 24% of the patient not on insulin therapy[111].

These rates increased to 84% and 53% respectively when the duration of diabetes mellitus increased to 15 - 19 years[111].

Classification of diabetic retinopathy is based in general on the severity of intraretinal microvascular changes and the presence or absence of retinal neovascularization.

Classification of diabetic retinopathy as presented by "Diabetic Retinopathy Study Research Group have been given in Table No.18.

The pathophysiological mechanism involved in non- proliferative diabetic retinopathy (NPDR) includes loss of retinal pericytes, increased retinal vascular permeability, alteration in retinal blood flow, and abnormal retinal microvasculature, all of which lead to retinal ischaemia.

Neovascularization in response to retinal ischaemia is the hallmark of proliferative diabetic retinopathy (PDR). These new vessels rupture easily leading to vitreous haemorrhage, fibrosis and ultimately retinal detachment[94].

RISK FACTORS FOR PROGRESSION OF DIABETIC RETINOPATHY.

1. Glycemic control : It has been found that the level of glycemic control is inversly proportional to the severity of diabetic retinopathy. Klein R. et al., Lloyd CE et al., Teuscher A. et al., have demonstrated that increased severity of diabetic retinopathy is associated with poor glycemia control[112,113,114].

DCCT (Diabetic Control and Complication Trial) have shown that the patients having HbA_{1c} of 10% have five fold greater risk of developing diabetic retinopathy as compared to the patients having 7% of HbA_{1c}[110].

Epidemiological analysis of the UKPDS (United Kingdom Prospective Diabetic Study) data showed a continuous relationship between the risk of microvascular complication and glycemia, such that for every percentage point decrease in HbA_{1c} (e.g. 9% to 8%), there was a 35% reduction in the risk of microvascular complication[110].

2. Hypertension : UKPDS has shown that intensive blood pressure control was associated with decreased risk of retinopathy progression.

Chaturvedi N. et al., in their study of `Blood Pressure Medication In Diabetic Retinopathy' have shown that there might be a specific benefit of Angiotensin converting enzyme (ACE) inhibition and blood pressure reduction, even in normotensive people, on the progression of diabetic retinopathy[115].

3. **Elevated Sr. Lipid Levels :** Increased total cholesterol and triglyseride were found to be associated with diabetic retinopathy. Chew E.Y et al., in ETDRS research and Klein B.E.K. et al., in WESDR (Wilconsin Epidemiologic study of Diabetic Retinopathy) have stated that elevated levels

of Sr.Cholesterol were associated with increased severity of retinal hard excudate[116,117]. Elevated Sr.TAG level were also associated with a greater risk for development of high risk proliferative diabetic retinopathy in ETDRS patients[185].

In a study in Pittsburg, elevated TAG and elevated LDL cholesterol were found to be associated with proliferative diabetic retinopathy[186].

Pathogensis of Early Diabetic Retinopathy

The pathway involved in the pathogenesis are the following.

1. **Polyol pathway :** Hyperglycemia leads to excessive production and accumulation of polyol, which has been shown to be important in the development of tissue in the lens and optic nerve[187,188].

Animal experiments suggest that an aldose reductase inhibitor could slow the development of diabetic retinopathy[189,190].

But, clinical trials in patient with type 2 diabetic mellitus have not yet demonstrated any retardation of progression of retinopathy, after administering aldose reeducates inhibitor "Sorbinil"[105,191].

2. **Non Enzymatic Glycation :** Non enzymatic glycation of proteins and DNA occurs during hyperglycemia, potentially altering enzyme activity and DNA integrity. This results in excessive cross - linking of proteins[90].

Aminoguanidine inhibits formation of AGE and has been reported to decrease the effects of diabetic mellitus on retinal blood flow, permeability, and other microvascular parameters in diabetic rats[91].

3. Protein kinase C : During period of hyperglycemia, PKC activity increase in the retina and retinal endothelial cell. PKC is known to affect vascular permeability, contractility and basement membrane synthesis and vascular cell proliferation.

RETINOPATHY SCREENING

According to the guidelines given by `American Diabetic Association' (ADA), scheme for retinopathy screening in diabetic population is as follows.

Patients with Type 1 diabetes mellitus should have an initial dilated and comprehensive eye examinations by an ophthalmologist or optometrist within 5 years after the onset of diabetes mellitus.

Patient with Type 2 diabetes mellitus should have initial and comphrensive eye examination by an ophthalmologist or optometrist shortly after diagnosis of diabetes mellitus followed by subsequent annual examination.

If eye is found to be normal during initial examination, follow up examination can be less frequent[120].

DIABETIC NEPHROPATHY

Diabetic nephropathy is major cause of diabetes mellitus related morbidity and mortality[51]. It is the leading cause of chronic kidney diseases in patients starting renal replacement therpay[107].

Diabetic nephropathy has been classically defined by the presence of proteinuria, >0.5 g/24 hr. This stage is referred to as overt nephropathy / clinical nephropathy / proteinuria or macroalbuminuria.

Incipient stage of diabetic nephropathy is when the albumin excretion in the urine is very less i.e. <300mg / 24 hr. This is termed as microalbuminuria.

The cutoff values adopted by the American Diabetes Association (timed, 24-h, and spot urine collection, for the diagnosis of micro and macroalbuminuria depicted in Table No.19.

In one study, only 30 - 45% of microalbuminuric patient have been reported to progress to proteinuria over 10 years[108].

Diabetic nephropathy develops at the most in 40% of patients with diabetes, even when high glucose levels are maintained for long period of time. This observation raised the concept that only a subset of patients have an increased susceptibility of diabetic nephropathy. Genetic susceptibility contributes to the development of diabetic nephropathy in patients with both type 1 and type 2 diabetes mellitus.

In addition to **`hyperglycemia', `hemodynamic insults'** and **`proteinuria'** perse has been identified as major mediator of renal damage in diabetics[109].

Figure No. 8, illustrates the mechanism of diabetic nephropathy.

Role of Hypertension in diabetic kidney damage

Hypertension plays a critical role in the progression of diabetic nephropathy. Levels of blood pressure closely related to the rate of decline in GFR[95].

Hyperglycemia induces vasodilatation with a marked decrease in afferent and to a lesser reduction in efferent arteriolar resistance. This leads to an increase in Glomerular capillary pressure levels and allows ready

transmission of any increase in systemic blood pressure to glomerular capillary circulation[95].

Increased intraglomerular pressure through increased mechanical stress and shear forces may damage the endothelial surface and disrupt the normal structure of glomerular barrier, eventually leading to mesangial proliferation, increased ECM production and thickening of glomerular basement membrane. These haemodynamic abnormality usually are associated with hypertrophic changes in the glomerulus.

Role of Proteinuria

Proteinuria of diabetic nephropathy is not only a complication of this disease but also a factor involved in it's pathogenesis.

Excessive tubular reabsorption of protein and the consequent accumulation of protein in tubular epithelial cells induces the release of vasoactive and inflammatory mediators, such as, TGF-β, endothelin - 1, osteopontin and macrophage chemotactic protein - 1.

These factors in turn lead to infiltration of mononuclear cells, causing injury to the tubulo-interstitium and ultimately renal scarring and insufficiency[109].

A vicious cycle is then established in which changes in renal hemodynamics either primary or in response to nephron loss induce further proteinuria, perpetuating a mechanism of interstitial scarring and progressive renal impairment.

Screening for diabetic nephropathy

Screening for diabetic nephropathy must be initiated at the time of diagnosis in patients with type 2 diabetes mellitus[118], since ~7% of them already have microalbuminuria at that time[119].

For Type I diabetes mellitus, first screening has been recommended at 5 years of diagnosis[118].

If microalbuminuria is absent, the screening must be repeated annually for both type 1 and type 2 diabetic patients[118].

As per guidelines of American Diabetes Association, the first step in the screening and diagnosis of diabetic nephropathy is to measure albumin in the spot urine sample, collected either as the first urine in the morning or at random, for example at the medical visit[118]. The results of albumin measurements in spot collection may be expressed as urinary albumin concentration (mg/dl) or as urine albumin to creatinine ratio (mg/g or mg/mmol).

MACRO VASCULAR COMPLICATION

CARDIOVASCULAR DISEASE

Cardiovascular disease is a prevalent and detrimental complication of diabetes mellitus.

Several observations highlight the high prevalence of cardiovascular disease in diabetes and the gravity of cardiovascular events in diabetic population.

According to Stamler J. et al., age-adjusted cardiovascular mortality is at least 2 fold higher in diabetic men than in nondiabetic subjects in the presence of any number of major risk factors[121]. Sprafka JM, et al., have stated the survival after MI is worse in diabetic men and women[122]. Haffuer SM et al., have found in their study that incidence of death from cardiovascular causes in diabetic subjects without a history of MI during 7 years follow up, was similar to the incidence observed in nondiabetic subjects with a history of MI[123].

Anderson AJ et al., and Quigley PJ et al., have shown that diabetic patients have greater occlusion of coronary arteries and a greater prevalence of multivessel disease[124,125].

Mooradian AD et al., and Thurman JE et al., have mentioned various factors which contribute accelerated atherosclerosis in diabetes mellitus[126,127]. These factors include excess prevalence of traditional risks such as Obesity, hypertension and dyslipidemia along with modification of lipoproteins and other key protein with glycation and oxidation, increased procoagulation and possibly the state of insulin resistance.

PERIPHERAL ARTERIAL DISEASE (PAD)

Peripheral arterial disease is most commonly a manifestation of systemic atherosclerosis in which the arterial lumen of the lower extremities become progressively occluded by atherosclerotic plaque[128]. It is a progressive condition, which may be symptomatic or asymptomatic.

Number of asymptomatic patients are found to be more as compared to the number of symptomatic patients[129].

Symptoms of PAD ranges in severity from intermittent claudication, (Pain relieved at rest) to critical limb ischemia (Pain at rest). Critical limb ischaemia if untreated, can lead to nonhealing wounds, gangrene, and eventual amputation.

Ongoing "Prevention of Progression of Arterial disease and Diabetes (POPADAD) study has demonstrated that 20% of the diabetic patients had, Ankle Brachial Index (ABI) value less than 0.91, which is clinically indicative of PAD[130].

Elhadal TA et al., in their study on diabetic population have found that 50% of diabetic patients were having PAD[130].

According to Hirsch AT et al., Elhadd TA et al., Murabito JM et al., advanced age, smoking and diabetes mellitus are strongly associated with PAD[130,131,132].

PAD is a powerful indicator of systemic atherosclerosis. According to one study conducted by Criqui, ME, et al., regardless of whether symptoms are evident, patients with PAD have an increased risk of subsequent MI and stoke and are 6 time more likely to die within 10 years than patient without PAD[133].

Type 2 DM patients with foot ulceration have macrovascular disease which impair skin oxygenation in such subjects. In absence of macrovascular disease, impaired nerve function may be associated with foot ulceration in Type 2 DM.

MAGNESIUM IN TYPE 2 DIABETES MELLITUS AND IT'S COMPLICATIONS : THEIR RELATION

Magnesium deficiency has been associated with many chronic diseases. Diabetes mellitus is one of them[136]. Gunn I.R. et al., Mather H.M. et al., have found upto 39% of out patient diabetics to be hypomagnesemic[134,135] while Tosiello found that nearly 25% of their diabetic outpatients were hypomagnesemic[137] . Low dietary intake of magnesium and loss of magnesium in urine via osmotic diuresis have been suggested as an explanation for lower level of magnesium in diabetic group. Lopez - Ridaura .R. et al., in their follow up study found that there was a significant inverse association between magnesium intake and diabetes mellitus risk[138]. Driziene Z et al., found that diurnal, overnight and 24 hrs, magnesium urinary excretion were significantly higher in diabetic subjects as compared to non-diabetic healthy subjects[139].

Wang J.L. et al have found in their study conducted in Thaiwan that there was an inverse association between plasma magnesium concentration and prevalence of diabetes mellitus. The risk of diabetes mellitus was elevated 3.25 times at plasma magnesium level < 0.863 mmol/L. Contrary to other studies they found that there is no association between diabetes and low dietary magnesium[140].

Low serum magnesium has shown to play an important role in pathogenesis of Insulin resistance. According to McCarty MF[141] magnesium can function as a mild, natural calcium antagonist. So in magnesium deficiency there is increased level of intracellular Calcium. This increased intracellular calcium may compromise the insulin responsiveness of adipocytes and skeletal muscles leading to insulin resistance[141].

Takaya J. et al., have suggested that magnesium is required for both proper glucose utilization and insulin signaling. Metabolic alteration in cellular

magnesium, which may play the role of a second messenger for insulin action, contribute to insulin resistance .[142].

Paolisso G. et al., Sgogran A et al., Nadder, J. et al., have reported that magnesium depletion perse impair insulin secretion and decreases peripheral insulin sensitivity and could contribute to diminished insulin effects[143,144,145].

Magnesium in Atherosclerotic Vascular Disease

Vitale J.J., et al., saw that experimental magnesium depletion is characterized by hypertriglyceridemia and hypercholesterolemia as well as atherosclerosis[146]. Serum concentration. of VLDL and LDL are elevated, whereas HDL is decreased. Decreased lipoprotein lipase activity as well as decreased lecithin cholesterol acyltransferase activity may be responsible for hyperlipidemia.[147].

This adverse lipid profile in hypomagnesemia is a strong risk factor for developing atherosclerosis.

Anetor JI et al., in their study on Nigerian population have found that type 2 diabetes mellitus patients have decreased serum magnesium level, probably suggesting lower antioxidant status in this condition. This may lead to greater chances of LDL-cholesterol oxidation, which is turn increases chances of Atherosclerosis[148].

Effect of Magnesium on vasculature and platelet function

In addition to increasing the likelihood of developing atherosclerosis, hypomagnesemia leads to increased platelet reactivity and vasospasm which is thought to be involved in genesis of myocardial infraction in hypomagnesaemia.

There are several theories for the mechanism of increased platelet reactivity and vasospasticity in hypomagnesaemia.

1. According to Altura B.M et al., hypomagnesaemia causes increased intracellular Ca^{++}. Increased intracellur calcium is cruicial for smooth muscle contraction and platelet - aggregation. So hypomagnesaemia increases smooth muscle contraction and platelet - aggregation[151].

2. Nadler J et al suggested that magnesium may be related to inhibition of the synthesis of thromboxane A2 and 12 HETE, eicosanoids thought to be involved in platelet aggregation[152,153]. So in magnesium depletion these eicosanoids are synthesized in increased amount leading to platelet aggregation.

   They also suggested that magnesium stimulate synthesis of PGI2, so in deficiency of magnesium PGI2 is not synthesized.

   Above mechanism leads to increased vasospasmability of coronary artery and could play a role of the onset of IHD.

   So, the lower serum magnesium in diabetic patients may be a explanation for the higher incidence of macrovascular complications like cardiovascular atherosclerosis and peripheral arterial disease is such patients.

Magnesium in peripheral vascular disease

Rodriguez - Moran et al found in their study that serum magnesium depletion is present and shows a strong relationship with fool - ulcers in subjects with Type 2 diabetes mellitus and foot - ulcers.[149].

Magnesium in retinopathy

Several contradictory reports are available regarding level of magnesium in microvascular complications of diabetes like diabetic retinopathy.

Harold et al., have found that patients with retinopathy have a lower mean plasma magnesium concentration than patients without retinopathy.

While according to Sheehan JP. the role of magnesium deficiency in microvascular complication has not yet been proved[150].

AIM OF THE STUDY

On reviewing the mineral magnesium and it's association with diabetes mellitus and various macro and microvascular complications of this disease, it was decided to analyse the above mineral in Type 2 diabetes mellitus and some of the complications of the disease with the following aim :

1. To determine the reference range of Sr.Mg for the study.

2. To determine the level of Sr.Mg in diabetes mellitus type 2 and to analyse whether it varied from the above reference range.

3. To determine the level of Sr.Mg in diabetic complications namely diabetic retinopathy, diabetic nephropathy, coronary atherosclerosis, and peripheral vascular disease and to analyse whether in these complications it varied from the reference range or from the corresponding level in Type 2 diabetes mellitus without any complication.

4. To correlate the level of Sr.Mg in the above conditions with the level of degree of control as determined by HbA_{1c} in Diabetes mellitus.

5. To determine whether a cutoff level for Sr.Mg can be obtained between apparently normal individuals and diabetics and between the latter level and the corresponding level in it's complications.

MATERIALS AND METHODS

This study was undertaken with the aim to determine Sr.Mg level in patients with Type 2 Diabetes Mellitus without it's associated complications and Type 2 Diabetes mellitus patients with it's various macro and micro vascular complications namely Coronary atherosclerosis, Peripheral vascular disease (Foot ulcer) and retinopathy, nephropathy respectively.

The study was conducted at Government General Hospital, Chennai on total of 120 subjects of age group 40 - 70 years; of whom 20 were apparently healthy and served as control. 20 apparently healthy subjects were formed of 10 male and 10 female. They were grouped as Category (1). Remaining 100 subjects formed the study group. The study group was constituted by 20 patients (10 male and 10 female) in each of the following categories.

Category 2 - Type 2 DM without it's associated complication.

Category 3 - Type 2 DM with coronary Atherosclerosis

Category 4 - Type 2 DM with peripheral vascular diseases (foot ulcer)

Category 5 - Type 2 DM with Retinopathy

Category 6 - Type 2 DM with Nephropathy

For the control group (category 1) the apparently healthy adults of 40 - 70 years were selected from staff of Madras Medical College and their relatives.

For category 2 (Type 2 DM without any of it's associated complication) patients were selected from outpatients clinic of Diabetology Department at

Government General Hospital, Chennai who came there for monitoring of Type 2 DM and treatment. They were diagnosed as having DM - Type 2 based on American Diabetic Association criteria of fasting hyperglycemia of ≥ 126 mg/dl after an overnight fast of 8 hours.

Those with inconsistent values of fasting glucose (110 mg/dl - 126 mg/dl) were diagnosed on the basis of OGTT. (WHO Criteria).

For Category 3 (Type 2 DM with coronary atherosclerosis) patients were selected from inpatients of Department of Diabetology, Department of Cardiology as well as from outpatient clinic of Department of Diabetology, GGH, Chennai. Diagnosis of coronary atherosclerosis was based on coronary. angiogram.

For Category 4 (Type 2 DM with Peripheral Vascular Disease) patients were selected from wards as well as from outpatient clinic of Department of Diabetology, GGH, Chennai, where they presented as foot - ulcer. Foot ulcer because of neuropathy was ruled out by history, clinical examination, `monofilament study' and `biothesiometry'.

For Category 5 (Type 2DM with Retinopathy) patients were selected from wards as well as from outpatient clinic of Department of Diabetology, GGH, Chennai. Diagnosis was based on Fundoscopic finding. NPDR and PDR both were included in the study.

For Category 6 (Type 2 DM with nephropathy) patients were selected from wards of Department of Diabetology, Govt. General Hospital, Chennai. Diagnosis of nephropathy was based on 24-hour urinary protein. Excretion of more than 500 mg / day of total protein in the 24 hours urine in diabetic patients were considered to be because of nephropathy.

Patients having more than one complication together (eg Type 2 DM with coronary atherosclerosis as well as retinopathy) were grouped under both the categories respectively.

While selecting subjects for the study group, following inclusion and exclusion criteria was adopted.

Inclusion Criteria	**Exclusion Criteria**
Consent	Recent H/o Diarrhoea, vomiting,
Age 40 - 70 yrs	Recent H/o Parentral fluid therapy Patient on drugs causing urinary loss of Mg e.g. Frusemide, Thiazide, diuretics. Alcoholic patients were not included in the study, as ethanol causes loss of Mg in the urine Smokers were not included in the study

For all the 120 subjects, 6 ml of peripheral venous blood was drawn under aseptic precaution from the anticubital vein, drawn blood was divided into 3 tubes as given below. Utmost care was taken to prevent hemolysis during the above procedure.

	Anticoagulant	**Amount of Blood (ml)**	**Investigations performed**
Eppendorff's Tube	EDTA	0.5	HbA_{1c}
Test Tube 1	NaF : Potassium Oxalate (1:3)	1.5	Pl- Glucose
Test Tube 2	Plane (Acid washed)	4	Serum - Magnesium Blood urea Sr- Creatinine

Blood in Test Tube 2 (Plane) was allowed to stand for one hour and Serum was separated by centrifugation after retraction of clot.

The separated serum was divided into 2 parts 0.5ml of serum was stored in clean, acid washed, dried eppendorff's tube at - 20°C, which was used for Sr.Mg estimation within 3 weeks of collection.

Remaining serum was utilised for estimation of parameters namely Bl - urea and Sr. Creatinine on the same day of collection.

ESTIMATION OF SERUM MAGNESIUM

Methodology

Sr.Magnesium was estimated using atomic absorption spectrophotometer in the Department of Animal Nutrition, Madras Veterinary College, Chennai.

Instrument

Atomic absorption spectrophotometer . " Model 3110 Perkin - Elmer".

Principle

Atomic absorption spectrophotmetry is based on the principle of absorption spectrophotometry. Here element to be analysed is dissociated from it's chemical bonds and placed in an unexcited or ground state (neutral atom). The ground state atom is capable of absorbing radiation at a very narrow bandwidth corresponding to it's own line spectrum. A hollow cathode lamp with the cathode made of the material to be analysed is used to produce a wavelength of light specific for the material. When the light from the hollow cathode lamp enters the flame, some of it is absorbed by the ground state atom in the flame, resulting in a net decrease in the intensity of the beam from the lamp. This process is referred to as atomic absorption.

Standarization of Instrument

Instrument was set using parameters given in Table No.20.

Accuracy of instrument was checked using working standard of concentration of 0.3 mg/dL. Working standard was prepared from stock

standard solution. Stock standard solution consisted of 1000 mg per litre of magnesium nitrate in nitric acid.

PROCEDURE

Serum sample was diluted one in ten using deionized water and reading was taken.

Final concentration of the sample was calculated using following equation.

$$\text{Concentration in mg/dL} = \frac{\text{Value in ppm x Dilution factor(10)}}{10^6} \times 100$$

ESTIMATION OF HbA_{1C}

Methodology

Cation - exchange Resin method was utilised for estimation of HbA_{1C} using "Monozyme's Glycohemin Kit".

Principle

Monozyme glycohemin kit is based upon the property of nonglycosylated hemoglobin to bind with a weak cation exchange resin leaving Glycated Haemoglobin (HbA_1) free in the supernatant.

Elimination of the labile schiff's base was achieved during hamolysis. The hemolysate is then mixed with a weakly binding cation exchange resin. The nonglycosylated haemoglobin binds to the resin leaving Glycated Haemoglobin free in the supernatant. The Glycated Haemoglobin percentage is determined by measuring the absorbance of Glycated Haemoglobin fraction and of the total Hb at 415 nm. This Glycated Haemoglobin constituted of HbA_{1a} + HbA_{1b} + HbA_{1c} . Value of HbA_{1c} is obtained from the value of HbA_1 from conversion table given in Table No.21.

Normal Range of HbA_{1c}

4.4 - 6.4%

Reagents and Consumable

Prefilled resin tubes - 10 x 2 ml

Lysing reagent - 5 ml

Resin separators - 10 Nos

Procedure

Step 1 - Hemolysate Preparation

250 µl lysing reagent was dispensed into required number of labeled tubes for different samples. 50µl of the well - mixed whole blood sample was placed into the appropriately labeled tube and mixed well. It was incubated for 5 minutes at room temperature to allow complete lysis of R.B.C.

Step 2 - Glycohemoglobin fraction separation

100 µl each of the hemolysates from the step 1 was added into respective resin tubes. Resin separator was inserted into each tube so that the rubber sleeve is approximately 1 cm above the liquid suspension of the resin. Tubes were placed on the rocker or rotator and mixed continuously atleast for 5 minutes. (Binding of nonglycosylated hemoglobin fraction to the resin requires atleast 5 minutes time). After this resin separator was pushed into the tubes until the resin is firmly packed to allow the supernatant to come into resin separator tube. Supernatant was directly aspirated into semiautoanalyser and reading was taken against distilled water at 415 nm.

Step 3 - Total hemoglobin fraction

5.0 ml of deionized water was dispensed into required number of properly labeled tubes. 20µl of each hemolysate from step I was added to the above tubes and mixed properly. Absorbance was read against distilled water at 415 nm.

Calculations

Results for the unknown samples were calculated as follows:

$$HbA_1 = \frac{\text{Absorbance of Glycohemoglobin}}{\text{Absorbance of Total Haemoglobin}} \times 4.61 \text{ (Assay factor)}$$

Values of HbA_{1c} was obtained from values of HbA_1 using Table No.21.

Estimation of blood urea, serum creatinine and plasma glucose was done using **urease method, Alkaline pictrate method** and **GOD / POD method** respectively.

RESULTS

The levels of the analysed biochemical parameters namely fasting plasma glucose, HbA_{1C}, Sr.Mg, Bl.urea, Sr.Creatinine, in 120 human subjects are tabulated in Table No.1 to 6 depending on the study group to which they belong.

Table No.1 enumerates the levels of the biochemical parameters in the control group formed by the 20 apparently healthy subjects. The first 10 subjects in the above table are apparently healthy males while the latter 10 subjects are apparently healthy females. The levels in apparently healthy male and apparently healthy female of the above table have been seggregated into tables namely 1a and 1b respectively.

Table 2 enumerates the biochemical parameter of Type 2 diabetics mellitus patients without any complications.

Table 3 enumerates biochemical parameters of Type 2 diabetes mellitus patients with angiogram proved coronary atherosclerosis.

Table 4 enumerates, biochemical parameters of Type 2 DM patients with Peripheral vascular disease who presented as foot ulcer.

Table 5 and 6 enumerates and biochemical parameters of Type 2 DM patient with retinopathy and nephropathy respectively.

The mean and SD for each parameter of the above tables which have been calculated are also shown in the respective tables. These mean values of

the parameters in the different groups are also depicted as histrogram in diagram No.1 to 5.

In order to find out whether the mean levels of the biochemical parameters in the entire control group (Table 1) can be utilised as the reference range for the study or considerable weightage has to be given for sex, the mean levels and the S.D. of the biochemical parameters in apparently health males and females (Table 1a and 1b) are compared with each other in Table No.7 Statistical significance has been ascertained for each parameter in the Table-7. Since none of the parameter of the study in the above table show any significant variation between sexes, the reference range selected for each parameter is the respective mean and S.D. obtained in Table 1 as in this table level of the parameters of all the control subjects irrespective of sex have been pooled together.

To find out statistically how far the levels of the biochemical parameters varied from the reference range in the different study groups, the mean and S.D. of each parameters in the different study groups. (Category 2-6) are compared with the obtained reference range in Table No.8 to 12.

Table 8 : Comparison of the levels of biochemical parameters in uncomplicated type 2 DM patients with that of reference range.

Table 9 : Comparison of the levels of biochemical parameters in type 2 DM patients with angiographically proved Coronary atherosclerosis (CAS) with that of reference range.

Table 10 : Comparison of the levels of biochemical parameters in type 2 DM patients with peripheral vascular disease presenting with foot ulcer with that of reference range.

Table 11 : Comparison of the levels of biochemical parameters in type 2 DM patients with retinopathy with that of reference range.

Table 12 : Comparison of the levels of biochemical parameters in type 2 DM patients with nephropathy with that of reference range.

To analyse how far the levels of biochemical parameters varied in the various complications of Type 2 diabetes mellitus from that of in the uncomplicated DM, the mean and S.D. of various biochemical parameters in each of the former group is compared with that of the latter in Table No.13 to 16

Table 13 : Comparison of the levels of biochemical parameters in type 2 DM patient with CAS. with that of uncomplicated group.

Table 14 : Comparison of the levels of biochemical parameters in type 2 DM patient with PVD presenting with foot ulcer with that of uncomplicated group.

Table 15 : Comparison of the levels of biochemical parameters in type 2 DM patient with retinopathy with that of uncomplicated group.

Table 16 : Comparison of the levels of biochemical parameters in type 2 DM patient with nephropathy with that of uncomplicated group.

The statistical significance between the levels of various biochemical parameter in all the comparison table was ascertained from the calculated "P" value which was arrived using the `student t test'

To determine how far Sr Mg varied with the severity of diabetes mellitus and the degree of control of the disease, Sr Mg. levels in the subjects, irrespective of grouping and as well with regard to grouping have been correlated with that of FPG and HbA_{1C} of the respective subjects in table No. 17 and 18. Karl-Person correlation coefficient between Sr.Mg and FPG in table number 17 shows negative and fair correlation in all the groups except in group consist of type 2 DM with retinopathy.

The table 18 shows the Karl-Pearson correlation coefficient between Sr.Mg. & HbA_{1C} arrived in the different groups & which have been seggregated into two subdivisions comprising of HbA_{1C} values less that 6.1% and equal or more than 6.1%.

This correlation between Sr.Mg.& FPG is also shown as scatter diagram in diagram No. 6 to 12. Similarly correlation between Sr.Mg. and HbA_{1C} in the entire 120 subjects irrespective of the grouping as well as in each of the study group is depicted as the scatter diagram in diagram No. 13 - 19.

To find out the cut off values for the Sr.Mg between control group and type 2 DM without any complication, line diagram is plotted in graph No.1. Graph No.2 shows the line diagram for Sr.Mg between uncomplicated type 2 DM and Type 2 DM with macrovascular complications namely CAS, PVD. (10 patients from each group of macrovascular complication are selected).

DISCUSSION

Discussion on the result is begun by considering the validity of the reference range obtained for the biochemical parameters of the study, which are 2.09 ± 0.30 mg/dl for Sr.Mg; 85.06 ± 15.82 mg/dl for fasting plasma glucose; 20.90 ± 5.31 mg/dl for blood urea 0.93 ± 0.22 mg/dl for Sr. creatinine; 5.46 ± 0.33% for HbA_{1C}.

Serum Mg level of 2.09 ± 0.30 mg/dl in the study correlates well with that of Maclean R, whose reference range is 1.7-2.4 mg / dl.[1] This level is also within the reference range of 1.7 - 2.5 mg/dl quoted by Pennel C Painter.[2]

Scrutinization of the reference range of other parameters of the study shows that they are well within the reference range quoted in standard text books and that of the kit methodology undertaken for their analysis.

Hence the acceptance of the mean values of the biochemical parameters obtained from the control group consisting of both males and famales, as reference range for the respective parameters is valid.

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Comparison of levels of various biochemical parameters of uncomplicated Type 2 DM with that of R.R. (Table 8) reveals that in uncomplicated Type 2 DM fasting plasma glucose is increased to HS levels (p=.000), HbA_{1C} is increased to MS level (p=.002) and Sr. Mg level is decreased to MS level (p=.001)

The HS increase of fasting plasma glucose (FPG) is an expected finding in view of the disease involved in the former group.

MS higher value of HbA_{1C} may be attributed to the poor compliance of the patient in the study group.

The decrease of Sr.Mg to MS levels in uncomplicated Type 2DM can be either a sequelae of Type 2 DM or can be an etiological factor of the disease as elaborated below :

1. Mg is an important factor for insulin secretion and insulin action. Mg depletion per se has been reported to impair insulin secretion as well as to reduce insulin sensitivity of the peripheral tissue.

2. The osmatic diuresis in Type 2 DM patients which is associated with loss of Mg in the urine leads to lower Mg value in the serum of such patients.

Mg impairing insulin secretion turns out to be an etiological cause, whereas osmotic diuresis depleting Mg turns out to be a sequelae of the disease.

***

Comparison of the levels of various biochemical parameters of Type 2 DM with macrovascular complications namely coronary atherosclerosis (CAS) and peripheral vascular disease (PVD) with that of reference range in Table No.9 and 10 reveals that while HbA_{1c} is increased to HS levels in type 2 DM with CAS (p = .000), it increases only to significant levels in type 2 DM with PVD (p=.036).

On the other hand, Sr.Mg is lowered to MS levels (p=.001) from the R.R in both the groups.

As HbA_{1c} signifies only the control of disease in the 3 months prior to the time of assessment, the elevation of HbA_{1c} is different in the two groups Probably the subjects in type 2 DM with PVD might have adhered more to the treatment than those belonging to the group of CAS.

As complications of type 2 DM normally sets only is long standing DM, the moderate decrease of Sr.Mg in both the groups of macrovascular complication is acceptable, since it's prevalence itself reveals the chronicity of the disease.

Absence of any statistically significant increase in fasting plasma glucose (FPG), inspite of HbA_{1c} showing statistical increase in macrovacular complication may be due to the temporary control of the glucose level on the day of assessment.

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Similarly when the levels of various biochemical parameters of type 2 DM with microvascular complications namely diabetic retinopathy and diabetic nephropathy is compared with that of the R.R, it is observed that HbA_{1c} values are statistically not different in type 2 DM with microvascular complication from the R.R. ($p= 1.000$ & $= 0.199$ in Table no 11 and 12 respectively).

On the other hand, MS decrease of Sr.Mg ($p=0.001$) is found both in Type 2 DM with retinopathy and in type 2 DM with nephropathy.

Absence of significant increase of HbA1c in type 2 DM with microvascular complications, and significant increase of HbA_{1c} in type 2 DM with macrovascular complications may be due to difference in following the treatment schedule by the subjects in different subsets.

The M.S. lower value of Sr.Mg in patients with type 2 DM with microvascular complication from the R.R. may once again be attributed to the chronicity of the disease.

Inspite of the normal Bl.urea level of 31.15 mg/dl in Type 2 DM with nephropathy, a HS increase has been obtained statistically for this parameter in

this complication on comparison with the R.R. As 31.15 mg/dl level of Bl.urea obtained in this complication is still within universally accepted R.R, it can be presumed that the subjects selected in this group might have had only mild nephropathy, which is also emphasized by the fact that Sr.creatinine level is also normal in this group. Moreover as the subjects of this group were inpatients of the hospital and were on strict diet regime with minimal protein intake, the normal level of blood urea is acceptable for this group.

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When the levels of various biochemical parameters in Type 2 DM with different complication are compared with the respective levels in uncomplicated type 2 DM in Table No.13,14,15,16, it is seen that Sr.Mg level in macrovascular complication is significantly decreased from that in uncomplicated type 2 DM ($p=0.05$ in both macrovascular complication), where as in type 2 DM with microvascular complication the difference does not exist ($p=1.000$ in both microvascular complication). This finding reveals an increase of the intensity of hypomagnesemia in only macrovascular complications of Type 2 DM to that of uncomplicated Type 2 DM.

As any macrovascular complication of DM is basically due to the development of atherosclerosis, it may be presumed that the further increase of intensity of hypomagnesemia observed in these complications may add up to the several etiological causes of altherosclerosis.

The theories attributing to the development of atherosclerosis in DM due to hypomagnesemia is given below:-

1. Mg is an antioxidant. Lowering of Mg level further in Type 2 DM patients puts such patients at higher risk of developing oxidized LDL which is very atherogenic and leads to higher degree of atherosclerosis.

2. Further, Mg depletion leads to greater increase than that in uncomplicated Type 2 DM, of triacylglycerol and total cholesterol in the body due to decreased lipoprotein lipase activity as well as decreased lecithin cholesterol acyl transferase activity[147].

It is a well known fact that increased level of the triacyglycerol and total cholesterol are atherogenic and greater their level, higher is the degree of atherogenesis.

The absence of any statistical difference in Sr.Mg level in Type 2 DM with retinopathy or nephropathy from the level in uncomplicated type 2 DM reveals that increase of the degree of hypomagnesemia is not a contributory factor for the development of these complication, which correlates well with the finding of Sheehan J.P. who had found that there is no role of further deficiency of Mg for the development of microvascular complications in type 2 DM[150].

The above finding is apt when we consider the pathogenesis of diabetic retinopathy and nephropathy as discussed previously on page 32 to 35. As per the review on these pages, in the pathogenesis of diabetic retinopathy and diabetic nephropathy Mg role has not been substantiated, moreover only polyol pathway, AGE and increased activity of protein kinase C are found to lead to the development of diabetic retinopathy; While in diabetic nephropathy it is hyperglycemia, AGE, polyol pathway, proteinuria and hypertension.

Absence of further increase in the intensity of hypomagnesemia in neprhopathy can also be explained on the following lines.

Nephropathy in various stages have various effects on Sr.Mg level.In early stages of nephropathy there is retention of magnesium while in advanced stages of nephropathy there is loss of Mg via urine.

As the subjects of type 2 DM with nephropathy selected in this study were all having early nephropathy, retention of Mg may be the reason for absence of hypomagnesemia in this group of patients.

So staging of nephropathy, which has not been done in this study is essential before Sr.Mg role is implicated in this complication.

FPG is lowered in all the diabetic groups with complication leading to statistically significant lowering of the parameter in all the above groups, except in CAS which can be attributed to the wide S.D obtained for this parameter in this group. Provided wide S.D. was not present in CAS, this group also would have shown S.S. decrease. The degree of statistically significance for FPG however varies between the groups.

Therefore it is inferred that there is statistically significant lowering of FPG in all the complication of Type 2 DM analysed, from it's level in uncomplicated Type 2 DM.

This lowering of FPG in diabetic complication can be attributed to the fact that these patients are more cautious in controlling the plasma glucose level by strictly adhering to the treatment schedule than those with uncomplicated Type 2 DM; for the former, patients would have started realizing the seriousness of the disease once the complication sets in.

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As hypomagnesemia has been found to be associated with Type 2 DM and a further statistical lowering of its level is seen in Type 2 DM with macrovascular complications; attempt has been made to arrive at a cut off level for Sr.Mg between control and uncomplicated Type 2 DM and that of the latter with macrovascular complication of Type 2 DM. For this purpose line graph have been drawn in Graph No.1, with the level of Mg in control and in patients of uncomplicated DM. Various cut off levels have been selected and their

calculated sensitivity / specificity, PPV / MVP are shown in Table No.19. 1.9 mg/dl of Sr.Mg which has a 80% sensitivity, 80% specificity, 80% positive predictive value 80% negative predictive value is found to be the most appropriate cut off between control and patients of uncomplicated DM i.e. levels of 1.9 mg/dl of Sr.Mg and below indicate Type 2 DM.

Similarly, graph has been plotted with levels in uncomplicated Type 2 DM and macrovascular complications of Type 2 DM in Graph No.2. The most appropriate cut off level between these entities as shown in Table No.20 is 1.5 mg/dl of Mg. i.e. level of 1.5 mg/dl and below of Sr.Mg indicate the presence of macrovascular complication namely coronary atherosclerosis and peripheral vascular disease in Type 2 DM. The above two cut off levels are also shown in the respective graphs.

When the Karl Pearson correlation coefficient between Sr.Mg and FPG (Table No.17) is analysed it is seen that there is a negative correlation between these two parameters in controls, uncomplicated type 2 DM, all groups of complications of Type 2 DM, and as well in the overall group. This negative correlation is fair in all the groups except DM with retinopathy which is poor. Negative correlation between Sr.Mg. and FPG may be because of loss of Mg in the urine via glycosuria in presence of hyperglycemia. So higher plasma glucose is associated with lower Mg in the serum.

Similarly, when the Karl - Pearson correlation coefficient between Sr.Mg and HbA_{1c} (both in 4.8 to 6.1 and >6.1 groups - Table No.18) is analysed it is seen that there in negative correlation between these two parameters in controls, uncomplicated type 2 DM, all groups of complications of Type 2 DM, and as well in the overall group. This negative correlation is fair to very good in various groups. Again negative correlation between Sr.Mg and HbA_{1c} can be attributed to loss of Mg via urine due to osmotic diuresis in presence of uncontrolled hyperglycemia.

CONCLUSION

From the analysis of the results obtained, in controls, Type 2 DM patients without complication and Type 2 DM with various macro and microvascular complication selected for the study, the following conclusions are made :

1. The reference range for S.Magnesium is 1.79 mg/dl to 2.39 mg/dl.

2. There is definite hypomagnesemia in Type 2 DM patients irrespective of the presence or absence of complication of Type 2 DM, when compared to control group.

3. Sr.Mg is statistically lower in macrovascular complications of Type 2 DM namely coronary atherosclerosis and PVD to that of its level in uncomplicated Type 2 DM.

4. The statistical lowering of Sr.Mg is absent between microvascular complication of Type 2 DM namely retinopathy and nephropathy and uncomplicated Type 2 DM.

5. Sr.Mg and HbA1c of <6.1% and >6.1% show negative correlation varying from fair to very good correlation in all the groups analysed.

6. Sr.Mg and FPG shows negative and fair correlation between all the groups except in Type 2 DM with retinopathy, where it shows negative and poor correlation.

7. 1.9 mg/dl is the cut off level for Sr.Mg between control group and Type 2 DM without any complication.

8. 1.5 mg/dl is the cut off level for Sr.Mg between Type 2 DM without complication and Type 2 DM with macrovascular complications namely CAS and PVD.

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SCOPE FOR FURTHER STUDY

1. Study can be done on the evaluation of Sr.Mg in Type 2 DM and it's various complication for larger number of patients in each group.

2. Other complications of Type 2 DM namely cerebrovascular accidents, neuropathy can also be included in study.

3. Evaluation of Sr.Mg in Diabetic nephropathy can be done according to stage of the nephropathy and also associated urine analysis for Sr.Mg can be done to evaluate the amount of Sr.Mg loss in urine.

4. Study can be extended to Type I DM.

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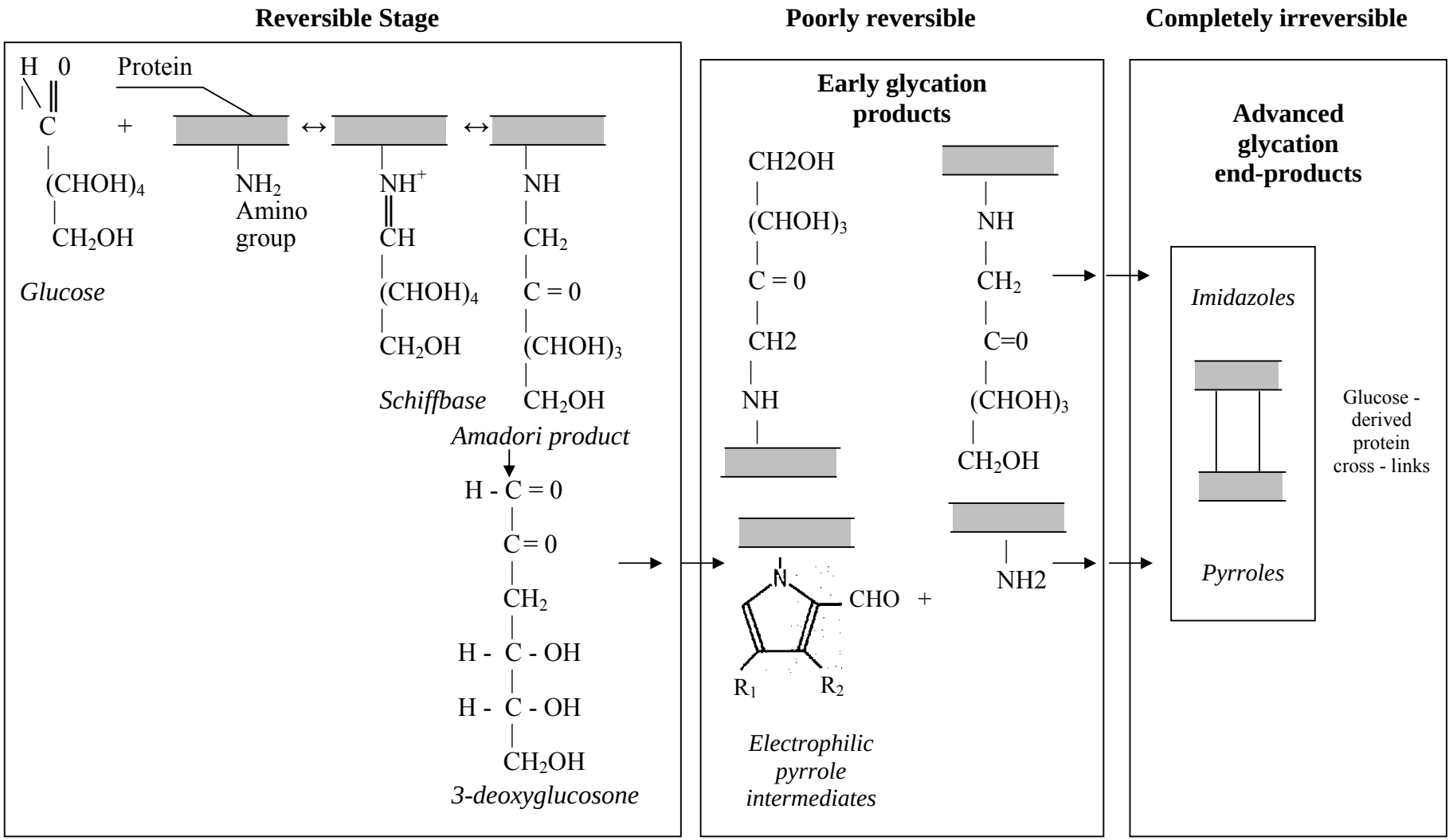
ABBREVIATIONS

AGE	-	Advanced glycosylation end product
ADA	-	American diabetic association
CVD	-	Cardivascular disease
CREAT	-	Creatinine
DCCT	-	Diabetes control and complication trial
DAG	-	Diacylglyserol
DM	-	Diabetes mellitus
ECM	-	Extracellular matrix
FPG	-	Fasting plasma glucose
GLUT	-	Glucose uptake and Transport
GAPDH	-	Glyseraldehyde 3 - phosphate dehydrogenase
HbA1c	-	Glycosylated hemoglobin
IRS	-	Insulin receptor substrate
IL-6	-	Interleukin - 6
IHD	-	Ischaemic heart disease
NEFA	-	Non esterified fatty acid
Mg	-	Magnesium
PKC	-	Protein kinase - C
PVD	-	Peripheral vascular disease
ROS	-	Reactive oxygen species
RDA	-	Recommended daily allowance
SOCS	-	Suppressor of cytokine signalling
TNF - α	-	Tissue necrosis factor - α
UKPDS	-	United Kingdom prospective diabetic study

FIGURE - 3 : FORMATION OF REVERSIBLE AND IRREVERSIBLE AGE



Courtesy : Text book of Diabetes 2. 3rd Ed., Blackwell Publishing

DIAGRAM - 6

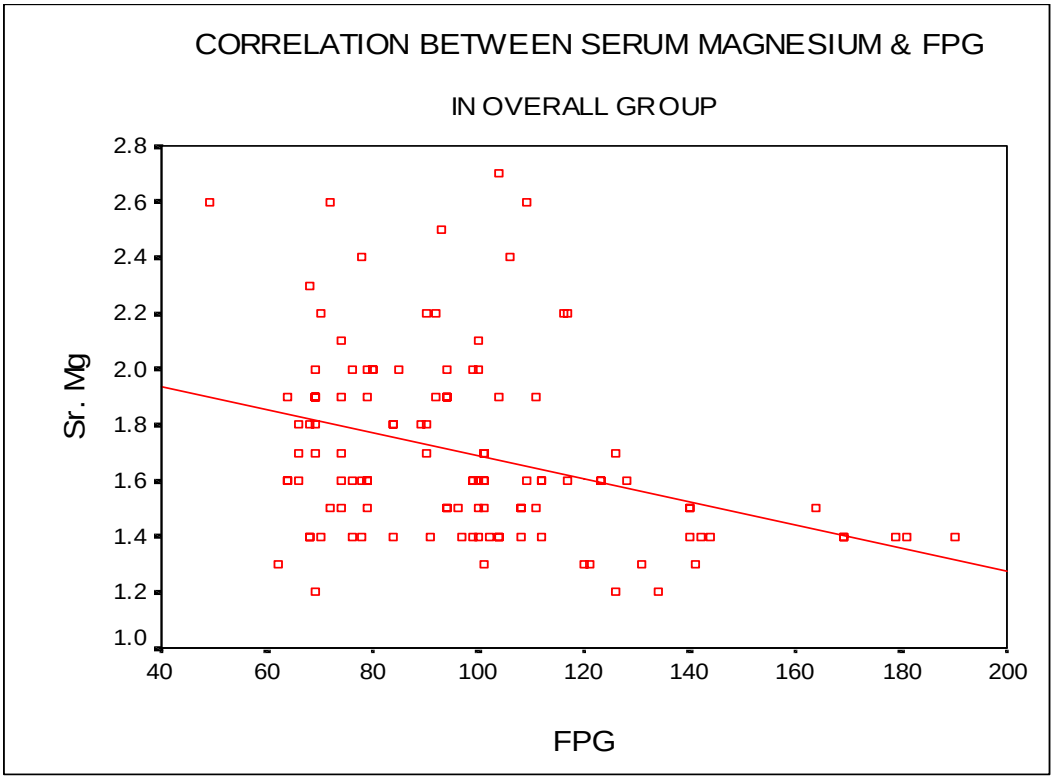
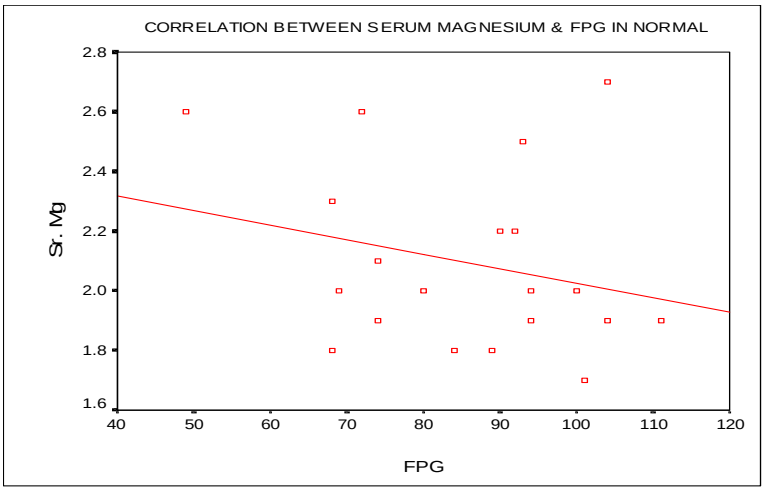

CORRELATION BETWEEN SERUM MAGNESIUM & FPG
IN OVERALL GROUP
Sr. Mg
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
40
60
80
100
120
140
160
180
200
FPG

DIAGRAM - 7



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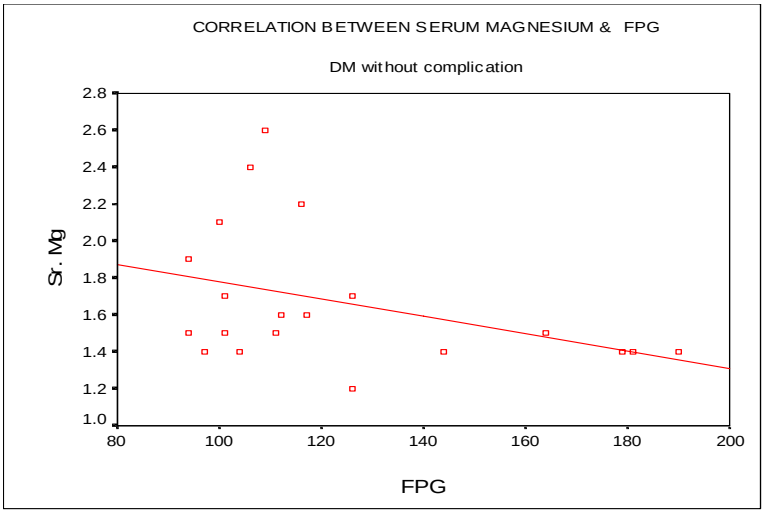

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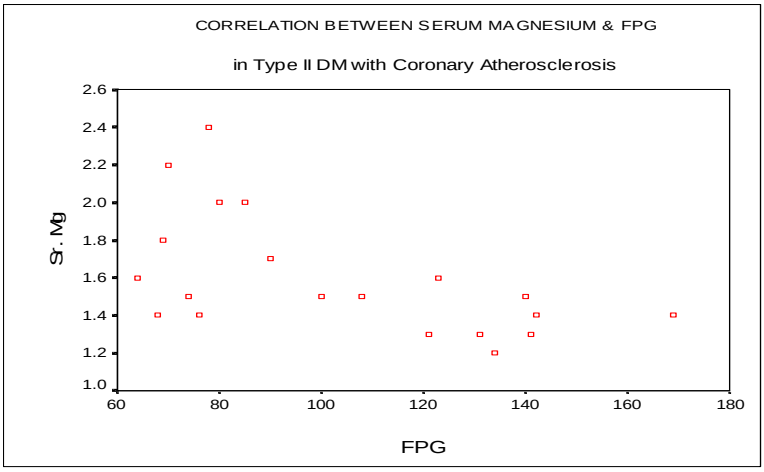

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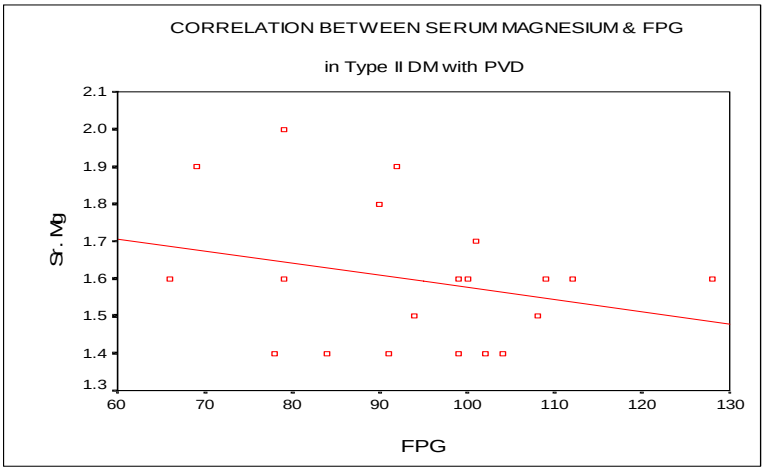

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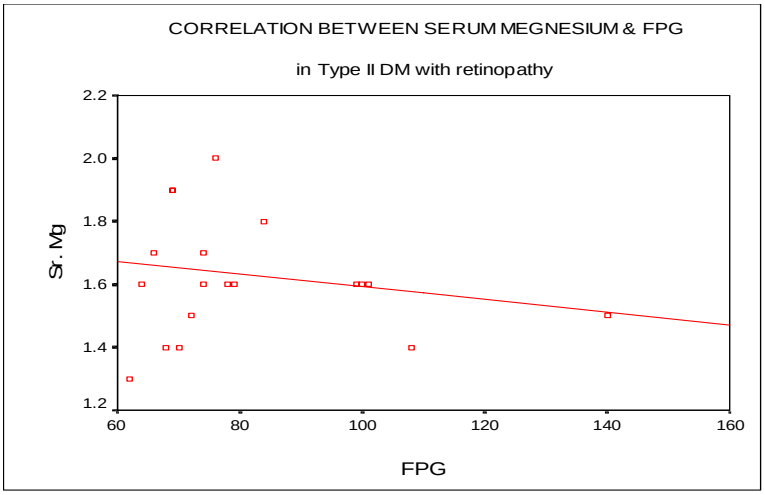

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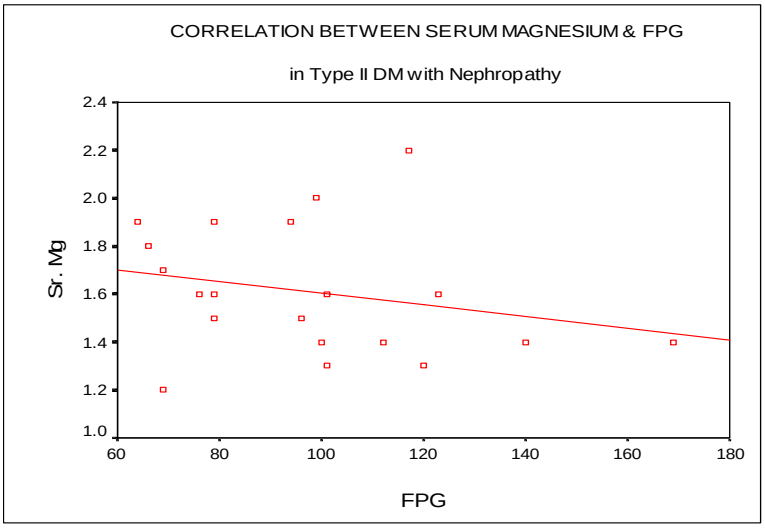

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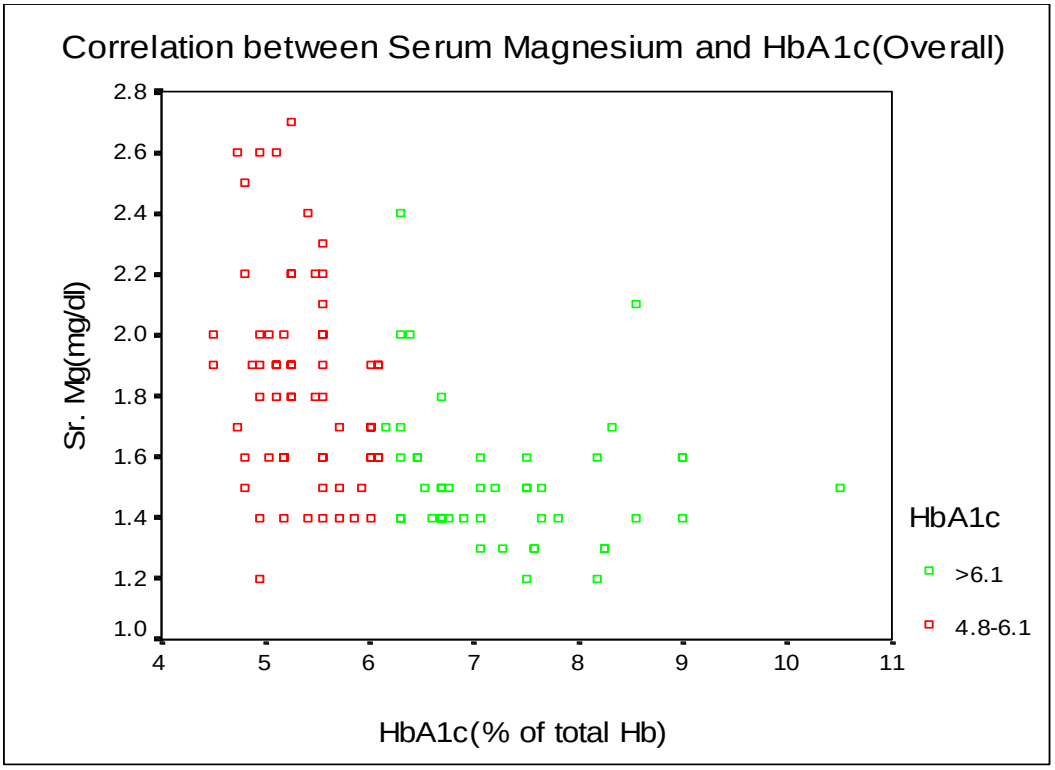
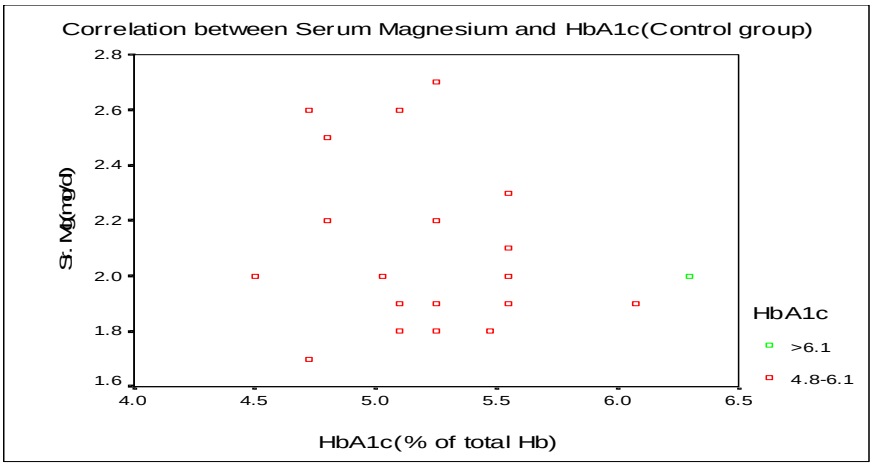

Correlation between Serum Magnesium and HbA1c(Overall)
Sr. Mg(mg/dl)
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
4
5
6
7
8
9
10
11
HbA1c(% of total Hb)
HbA1c
>6.1
4.8-6.1

DIAGRAM - 14



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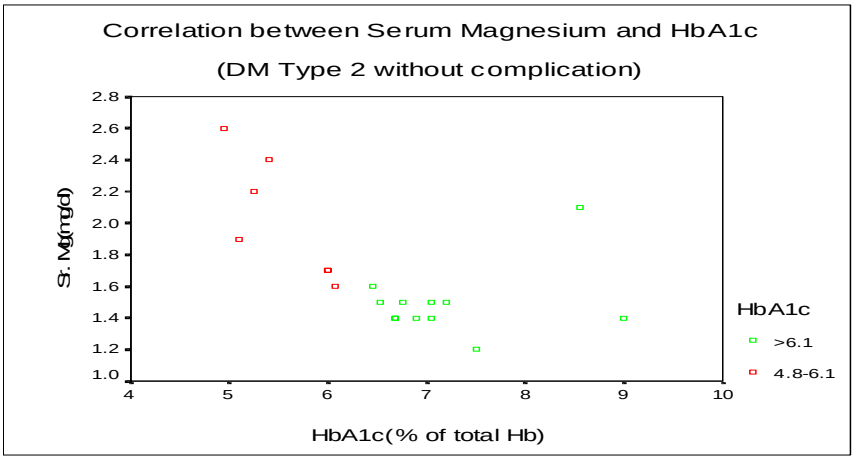

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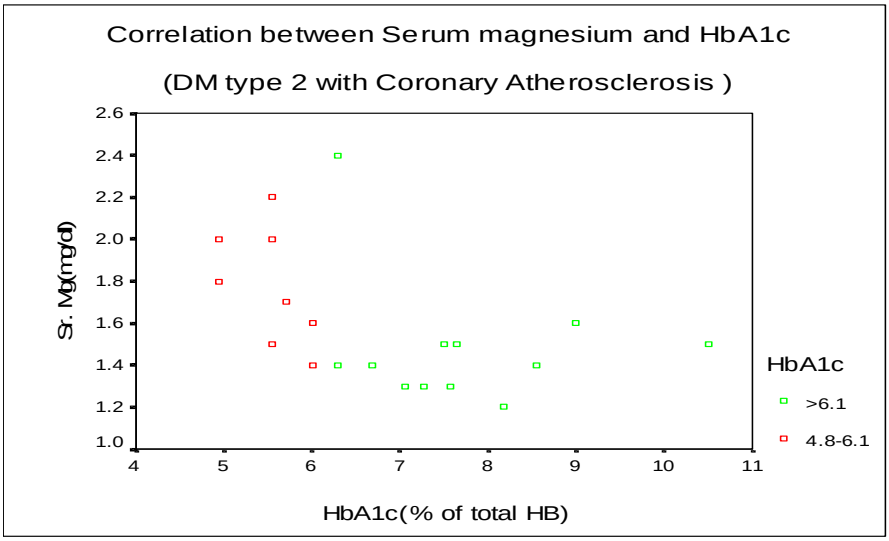

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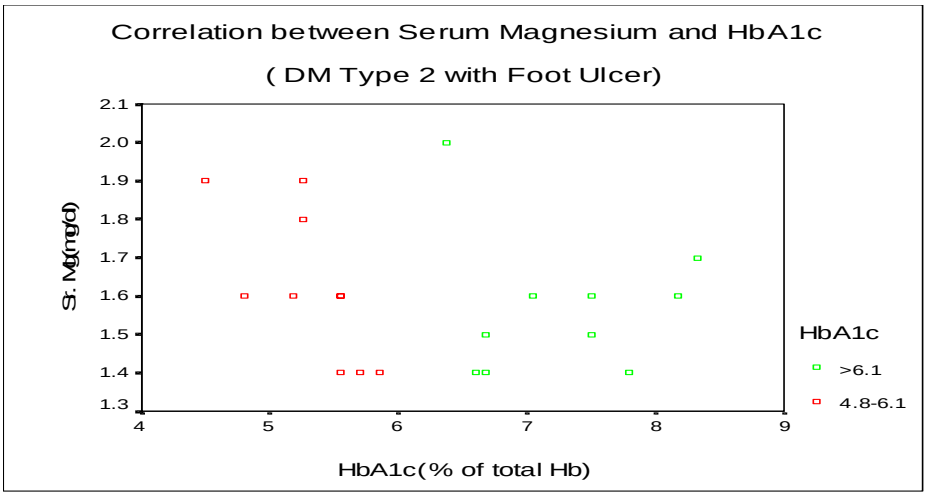

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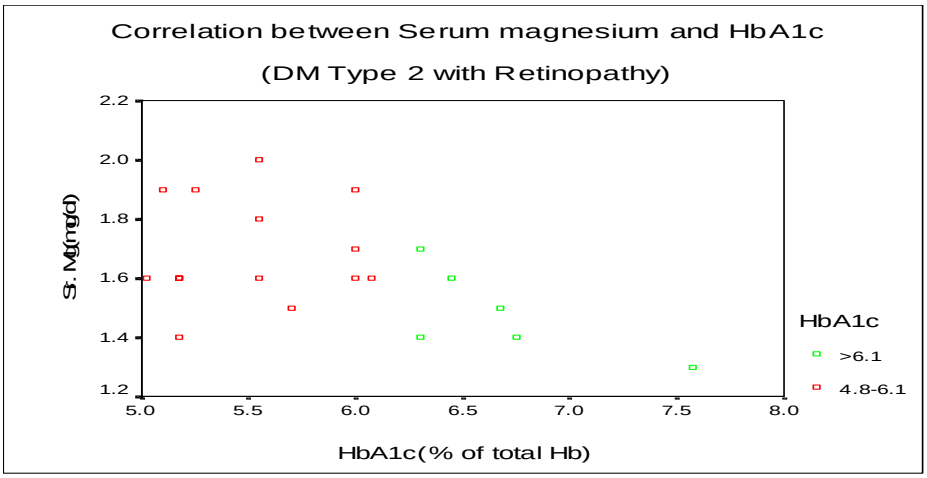

DIAGRAM - 19